Please amend the Brief Description of the Figures to describe Figures 7A, 7B and 7C and 8A and 8B as shown on the attached new and marked up versions of pages 10 and 11, presented in Appendix C.

REMARKS

A. Objections

The Examiner had objected to claim 72 for depending from non-elected claim 68. Applicant submits that the above amendment to claim 72 should serve to obviate this objection.

The Examiner had objected to the title as being non-descriptive. Applicant submit that the above amendment to the title should serve to obviate this objection .

The Examiner had objected to page 8 of the specification as line 25 is blurred. A clean copy of new page 8 is submitted with this response in Appendix D and Applicants request removal of the objection to page 8.

The Examiner had objected to the description of the drawings because the specification does not include a description for each figure of the drawings. Applicant submits the above amendment to the Brief Description of the Figures serves to address this objection by specifically referring to Figures 7A, 7B and 7C as well as Figures 8A and 8B.

B. Status of the Claims

In response to the original restriction requirement in this application Applicants elected Group I comprising claims 59-63 and 72-73. Concurrent with the election Applicants submitted a Preliminary Amendment changing the dependency of claims 64 and 82 to elected linking claim 59. Consideration of claims 64-71, 74-81 and 82-96 linked to claim 59 is requested on allowance of claim 59. Confirmation of entry of the Preliminary Amendment is respectfully requested. A copy of this Preliminary Amendment is included in Appendix E.

C. Rejection Under 35 U.S.C. §112, First Paragraph – Written Description

Claims 59-63 and 72-73 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey possession of the invention, at the time the application was filed, to one of skill in the art.

The Examiner cites U.C. v. Lilly, (43USPQ2d 1398 (Fed. Cir. 1997), indicating that the written description "requires a precise definition such as by structure, formula [or] chemical

name, of the claimed subject matter sufficient to distinguish it from other materials." Applicants would like to point out that genes encoding enzymes that can elevate the level of proline were available in the art at the time of filing. Verma *et al.* in U.S. Patent 5,344,923, filed September 29, 1992 (Verma I) discloses the isolation of a mothbean cDNA clone encoding a bifunctional enzyme, delta^1-pyrroline-5-carboxylate synthetase, which is involved in the biosynthesis of proline in plants. The sequence of a soybean homologue of delta^1-pyrroline-5-carboxylate synthetase was disclosed in 1992 by Hu *et al.* in *Proceedings National Academy of Science* (*PNAS* 89:9354-9358); Hu *et al.* also disclose that the enzyme catalyzes the first two steps in proline biosynthesis in plants. Clearly a person of ordinary skill in the art would have known at that time of Applicants invention of useful gene sequences involved in the synthesis of proline. Applicants respectfully submit that the availability of such gene sequences as common knowledge obviates the rejection under 35 U.S.C. §112, first paragraph. Similar disclosure was deemed sufficient to describe an analogous invention related to glycine betaine in the related application which issued as U.S. Patent 6,281,411. Reconsideration and withdrawal of this outstanding Section 112 rejection for lack of written description is respectfully requested.

D. Rejection Under 35 U.S.C. §112, First Paragraph – Enablement

Claims 59-63 and 72-73 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. In particular the Examiner takes issue with an alleged failure to disclose the identification or isolation of a particular gene and/or plant comprising recombinant DNA encoding an enzyme involved in proline synthesis.

However, the Applicants argue that the claims are fully enabled by the specification. No evidence has been put forth by the Examiner sufficient to doubt enablement. Because Applicants invention is not limited to a particular gene, the above-described deficiency is not relevant to enablement. Applicants have enabled a person of ordinary skill in the art to make transgenic plants using mannitol producing enzymes (see US Patent 5,780,709) and glycine betaine producing enzymes (see U.S. Patent 6,281,411). Applicants have equated mannitol, glycine betaine and proline as osmoprotectants of value when over-expressed in transgenic plants.

In this regard, Applicants note that the Examiner has apparently placed the burden on Applicant to affirmatively establish enablement. However, the examiner has failed to meet the burden of establishing a *prima facie* case of lack of enablement by any showing that equivalence

of proline with mannitol for the intended effect is not established. An unsupported allegation of lack of enablement does not meet the PTO's burden under 35 U.S.C. § 112, first paragraph, "[o]therwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." *In re Marzocchi*, 169 U.S.P.Q. 367, 369-70 (CCPA 1971).

As stated above, sequences for particular genes involved proline biosynthesis were known in the art. In addition, Applicants also note that proline has long been identified as playing a role in plants under water deficit. Barnett *et al.* (*Plant Phys.* 41:1222, 1966) describe that under water deficit, significant increases in certain amino acid pools, such as proline, are observed. Wyn Jones and Storey (Physiology and Biochemistry of Drought Resistance in Plants, Chapter 9, p 171-204, Academic Press, Australia, 1981) note that increased proline accumulation is observed in barley subject to water or salt stress. McCue and Hanson (*TIBTECH*, 8:358-362, 1990) specifically mention the amino acid proline as an osmoprotectant found in diverse organisms. Van Rensberg *et al.* (*J. Plant Physiology*, 141:1880194, 1993) discuss their observations of increased proline accumulation in drought-resistant tobacco cultivars, where a substantial amount of proline was found to accumulate in the drought-resistant cultivars compared to the drought-sensitive cultivars.

Applicants have previously taught the use of increased mannitol and increased glycine betaine as a means for imparting water stress tolerance to transgenic monocot plants (see US Patents 5,780,709 and 6,281,411, respectively). Applicants' success in using the *mtlD* gene to impart drought tolerance to a monocot in fact reduces the unpredictability in the art with respect to other genes that are known to function in plants.

Together, the knowledge of proline accumulation in plants in response to drought stress, as well as the knowledge of sequences from soybean and mothbean involved in the synthesis of proline, would have enabled one skilled in the art at the time of filing to prepare plants expressing a proline biosynthesis gene and expect that an increase in proline would lead to increase water stress tolerance. The consistent report of increased proline in plants in response to water stress seems a good indicator of expecting a plant with increased proline to exhibit water stress tolerance. Applicants submit that the above remarks illustrate enablement sufficient for a person of ordinary skill in the art to practice the claimed invention. Reconsideration and

withdrawal of this outstanding Section 112 rejection for lack of enablement is respectfully requested.

E. **First Rejection Under 35 U.S.C. §112, Second Paragraph – Indefiniteness**

Independent claim 61 and dependent claims 62-63 stand rejected under 35 USC 112, second paragraph, as being indefinite for the use of the term "increased", citing that this is a relative term lacking comparative basis.

Applicants point out that a definition of "increased" offered on page 6, lines 26-30, is, literally comparative, wherein:

> "The enzyme encoded by the DNA segment is expressed in the cells of the differentiated plant in an amount effective to increase the mannitol content in the cells of the differentiated plant relative to the mannitol content in the cells of an untransformed differentiated monocot plant." (emphasis added)

In addition, Applicants further define "increased" on page 8, line 24 to page 9, line 6, wherein:

> "As used herein, "substantially increased" or "elevated" levels of an osmoprotectant in a transformed plant cell, plant tissue, plant part, or plant, are greater than the levels in an untransformed plant cell, plant part, plant tissue, or plant, i.e., one where the genome has not been altered by the presence of a preselected DNA sequence. In the alternative, "substantially increased" or "elevated" levels of an osmoprotectant in a water-stressed transformed plant cell, plant tissue, plant part, or plant, are levels that are at least about 1.1 to 50 times, preferably at least about 2 to 30 times, and more preferably about 5-20 times, greater than the levels in a non-water-stressed transformed plant cell, plant tissue, plant part or plant.
>
> For example, the levels of mannitol in a monocot plant transformed with a preselected DNA sequence encoding an enzyme which catalyzes the synthesis of mannitol, are compared to the levels in an untransformed plant." (emphasis added)

Thus, as used in the instant invention, "increased" is defined in terms relating the expression of a preselected DNA resulting in an increase in an osmoprotectant in a transgenic plant, exemplified by mannitol, in relation to the amount of the osmoprotectant in a non-transgenic plant. By this definition, Applicants believe that "increased" is defined and definite and reconsideration and withdrawal of the Section 112, second paragraph rejection is respectfully requested.

F. **Second Rejection Under 35 U.S.C. §112, Second Paragraph – Indefiniteness**

Claim 73 stands rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of the term "transgenic seed." The Examiner argues that the seed may contain

transgenes in addition to those used in the method of claim 68. Whatever other characteristics the plant may have in addition to those required by the claim do not make the claim indefinite.

Applicants point out however, that claim 72, from which claim 73 depends, has been amended to depend from claim 64. Claim 73 is directed to the seed of definite transformed monocot plant. Reconsideration and withdrawal of this Section 112, second paragraph, rejection is respectfully requested.

G. **Rejection Under 35 U.S.C. §102(e), Anticipation**

The Examiner rejects claims 59-61 under 35 USC 102(e) as being anticipated by Verma *et al.* U.S Patent No. 5,639,950, issued June 17, 1997, filed June 29, 1994 as a CIP of an application with an effective filing date of September 29, 1992 (hereinafter Verma II) . Reference is made to the original Verma *et al.* application which was filed on September 29, 1992 and issued as U.S. Patent No. 5,344,923 (hereinafter Verma I).

Applicants' application claims priority to August 25, 1993 as a divisional application of U.S. application Serial No. 08/599,714 filed January 19, 1996 (now U.S. Patent No. 6,281,411) which is a continuation-in-part application of currently pending U.S. application Serial No. 08/113,561, filed August 25, 1993, the grandparent application which disclosed transgenic monocot plants with drought resistance can be achieved by expressing genes encoding a variety of osmotically active metabolites including proline.

Applicants respectfully traverse this rejection because the Verma I does not anticipate Applicants' claimed subject matter and the Verma II patent is effective only as of its June 1994 filing date for disclosure of "corn". Neither "corn" nor synonymous terms "monocot" or "maize" appear in the description of Verma I. More particularly, Applicants' claims are drawn to a transformed monocot plant which is substantially tolerant or resistant to a reduction in water availability wherein the transgenic plant comprises a transgene encoding an enzyme which catalyzes the synthesis of proline. Verma I merely discloses the sequence of delta^1-pyrroline-5-carboxylate synthetase making the sequence available to those of ordinary skill in the art who might discover a use for it, e.g. as Applicants have discovered a use in producing transgenic monocots expressing proline for drought resistance.

In addition, neither Verma I nor Verma II are enabling for the transformation of monocots. Applicants thus argue that neither Verma I nor Verma II constitute a 35 USC 102(e) bar to the present application.

In view of the above remarks Applicants respectfully request reconsideration and withdrawal of this Section 102(e) rejection.

H. Rejection Under 35 U.S.C. §103(a), Obviousness

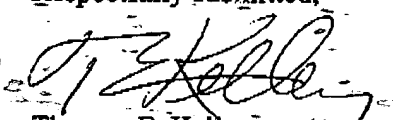
The Examiner rejects claims 59-63 and 72-73 under 35 U.S.C. 103(a) as being unpatentable over Verma II (described above) in view of Rayapati *et al.* (Plant Physiology, 1989, 91:581-586) and in light of Applicant's admitted prior art.

The teachings of Verma II are discussed above and, as noted by Examiner, do not disclose the use of an amino terminal chloroplast transit peptide. Rayapati *et al.* is cited for its finding that delta^1-pyrroline-5-carboxylate synthetase is located in the chloroplast of peas.

Applicants respectfully traverse this rejection and submit that the Examiner has not made a *prima facie* case of obviousness for claims directed to transformed monocots having DNA which encodes an enzyme which catalyzes the synthesis of the osmoprotectant proline, regardless of the presence of an amino terminal chloroplast transit peptide. While Verma II claim a number of transgenic plant types, including maize, they are not enabling for these plants. Rayapati *et al.*, is a biochemical paper which does not teach transgenic plants at all, and thus does not cure of the defect of Verma II in failing to teach how to make transformed monocot plants. Nor do the combined references teach fertile, transformed corn. What in these references would motivate a person of ordinary skill in the art to attempt to transform corn with no teaching or motivation discussed or implied in either reference? Applicants respectfully submit that that there is no support for a *prima facie* obviousness rejection of claims 59-63 and 72-73 over Verma II in light of Rayapati *et al.* Reconsideration and withdrawal of this Section 103 rejection is respectfully requested.

Applicants submit that claims 59-63 and 72-73 are patentable over the art of record as discussed in this response. An early favorable action with allowance of these claims is earnestly solicited.

Respectfully submitted,



Thomas E. Kelley
Registration Number 29,938
Attorney for Applicants
860 572 5274

APPENDIX A
MARKED UP COPY Claim 72

72. (Amended) A transformed monocot plant regenerated from the transformed plant cells obtained by the method of claim [68] 64.

APPENDIX A
CLEAN COPY Claim 72

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72. (Amended) A transformed monocot plant regenerated from the transformed plant cells obtained by the method of claim 64.

APPENDIX B
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TRANSGENIC MAIZE WITH INCREASED [MANNITOL] PROLINE CONTENT

Cross-Reference to Related Applications

This application is a divisional application of U.S. application Serial No. 08/599,714, filed January 19, 1996, allowed, which is a continuation-in-part application of currently pending U.S. application Serial No. 08/113,561, filed August 25, 1993, which are incorporated by reference herein.

Background of the Invention

Unpredictable rainfall, increases in soil salinity, and low temperature at the beginning or end of the growing season often result in decreased plant growth and crop productivity. These three environmental factors share at least one element of stress and that is water deficit or dehydration.

Drought is a significant problem in agriculture today. Over the last 40 years, for example, drought accounted for 74% of the total U.S. crop losses of corn (Agriculture, U.S. Department of, 1990. Agricultural Statistics. US Government Printing Office, Washington, D.C.). To sustain productivity under adverse environmental conditions, it is important to provide crops with a genetic basis for coping with water deficit, for example by breeding water retention and tolerance mechanisms into crops so that they can grow and yield under these adverse conditions.

When the rate of aspiration exceeds that of water uptake or supply, water deficit occurs and wilting symptoms appear. The responses of plants to water deficits include leaf rolling and shedding, stomata closure, leaf temperature increases, and wilting. Metabolism is also profoundly affected. General protein synthesis is inhibited and significant increases in certain amino acid pools, such as proline, become apparent (Barnett et al., *Plant Physiol.* 41, 1222 (1966)). During these water deficit periods, the photosynthetic rate decreases with the ultimate result of loss in yield (Boyer, J. S., In: *Water deficits and plant growth,* T. T. Kozlowski (ed.)., Academic Press, New York., pp. 154-190 (1976)). If carried to an extreme, severe water deficits result in death of the plant.

APPENDIX B
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When the rate of aspiration exceeds that of water uptake or supply, water deficit occurs and wilting symptoms appear. The responses of plants to water deficits include leaf rolling and shedding, stomata closure, leaf temperature increases, and wilting. Metabolism is also profoundly affected. General protein synthesis is inhibited and significant increases in certain amino acid pools, such as proline, become apparent (Barnett et al., *Plant Physiol.* 41, 1222 (1966)). During these water deficit periods, the photosynthetic rate decreases with the ultimate result of loss in yield (Boyer, J. S., In: *Water deficits and plant growth,* T. T. Kozlowski (ed.)., Academic Press, New York., pp. 154-190 (1976)). If carried to an extreme, severe water deficits result in death of the plant.

APPENDIX C
Marked up version of pages 10-11

superior osmotic potential during a water deficit relative to the corresponding, i.e., substantially isogenic, recurrent inbred plant.

As used herein, an "exogenous" gene or "recombinant" DNA is a DNA sequence or segment that has been isolated from a cell, purified, and amplified.

As used herein, the term "isolated" means either physically isolated from the cell or synthesized in vitro in the basis of the sequence of an isolated DNA segment.

As used herein, a "native" gene means a DNA sequence or segment that has not been manipulated in vitro, i.e., has not been isolated, purified, and amplified.

As used herein, "altered" levels of an osmoprotectant in a transformed plant, plant tissue, plant part, or plant cell are levels which are different, preferably greater, than the levels found in the corresponding untransformed plant, plant tissue, plant part, or plant cells. In the alternative, altered levels of the osmoprotectant in a backcross converted inbred transformed plant are different, preferably greater, than the levels found in the corresponding recurrent inbred plant.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. A schematic diagram of plasmid pDPG451.

FIG. 2. A schematic diagram of plasmid pDPG165.

FIG. 3. A schematic diagram of plasmid pDPG480.

FIG. 4. A schematic diagram of plasmid pDPG493.

FIG. 5. A schematic diagram of plasmid pDPG586.

FIG. 6. A schematic diagram of plasmid pDPG587.

[FIG. 7. A time course of leaf osmotic potential values collected from a population of transgenic maize plants. All plants were derived from AT824 cells bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos-containing medium. (A) S80HO-5201, (B) S80HO-5205, and (C) S80HO-5208.]

FIG. 7A. A time course of leaf osmotic potential values collected from a population of transgenic maize plants comprising event S80HO-5201. All plants were derived from AT824 cells bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos-containing medium.

FIG. 7B. A time course of leaf osmotic potential values collected from a population of transgenic maize plants comprising event S80HO-5205. All plants were derived from AT824 cells

bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos-containing medium.

FIG. 7C. A time course of leaf osmotic potential values collected from a population of transgenic maize plants comprising event S80HO-5208. All plants were derived from AT824 cells bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos-containing medium.

[FIG. 8. Leaf temperature data from Glufosinate® sensitive (mtlD negative) and resistant (mltD positive) plants grown under water stress conditions in the field.]

FIG. 8A. Leaf temperature data from Glufosinate® sensitive (mtlD negative) and resistant (mltD positive; low expressor) plants grown under water stress conditions in the field.

FIG. 8B. Leaf temperature data from Glufosinate® sensitive (mtlD negative) and resistant (mltD positive; high expressor) plants grown under water stress conditions in the field.

DETAILED DESCRIPTION OF THE INVENTION

The identification and characterization of plants that are resistant or tolerant to water deprivation has long been a goal of agronomy. However, it has not been possible to accomplish the identification and isolation of genes that can provide resistance or tolerance to water stress. The insertion of such genes into monocots has the potential for long-term improvement in, and expansion of, agriculture world-wide.

The ability of a plant to adapt to changes in water and salt concentrations is dependent on the ability of the plant to osmotically adjust its intracellular environment by altering the concentration of osmoprotectants within the cells of the plant. These osmoprotectants include, but are not limited to, various sugar molecules, such as monosaccharides, disaccharides, oligosaccharides, polysaccharides, sugar alcohols, and sugar derivatives. Thus, to provide a plant that is tolerant or resistant to a reduction in water availability, a preselected DNA segment or "gene" or "transgene" encoding an enzyme which catalyzes the synthesis of a particular osmoprotectant can be introduced into the genome of the plant. The osmoprotectant may be one that is not normally synthesized by the plant, but one which can be synthesized from a substrate that is abundant in the cells of the plant after the introduction of the preselected DNA segment. In the alternative, the osmoprotectant may be one that is naturally synthesized by the plant but the levels of the osmoprotectant in the plant are insufficient to render the plant tolerant to a reduction in water availability.

The accumulation of a non-naturally occurring osmoprotectant in a plant, plant cell, plant part, or plant tissue, could result in a detrimental effect because the substrate employed to synthesize the osmoprotectant is being depleted and a non-naturally occurring product is produced, which most likely would not be degraded. Moreover, a single introduced preselected DNA segment in the

APPENDIX C
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superior osmotic potential during a water deficit relative to the corresponding, i.e., substantially isogenic, recurrent inbred plant.

As used herein, an "exogenous" gene or "recombinant" DNA is a DNA sequence or segment that has been isolated from a cell, purified, and amplified.

As used herein, the term "isolated" means either physically isolated from the cell or synthesized in vitro in the basis of the sequence of an isolated DNA segment.

As used herein, a "native" gene means a DNA sequence or segment that has not been manipulated in vitro, i.e., has not been isolated, purified, and amplified.

As used herein, "altered" levels of an osmoprotectant in a transformed plant, plant tissue, plant part, or plant cell are levels which are different, preferably greater, than the levels found in the corresponding untransformed plant, plant tissue, plant part, or plant cells. In the alternative, altered levels of the osmoprotectant in a backcross converted inbred transformed plant are different, preferably greater, than the levels found in the corresponding recurrent inbred plant.

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FIG. 5. A schematic diagram of plasmid pDPG586.

FIG. 6. A schematic diagram of plasmid pDPG587.

FIG. 7A. A time course of leaf osmotic potential values collected from a population of transgenic maize plants comprising event S80HO-5201. All plants were derived from AT824 cells bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos containing medium.

FIG. 7B. A time course of leaf osmotic potential values collected from a population of transgenic maize plants comprising event S80HO-5205. All plants were derived from AT824 cells bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos containing medium.

FIG. 7C. A time course of leaf osmotic potential values collected from a population of transgenic maize plants comprising event S80HO-5208. All plants were derived from AT824 cells

bombarded with pDPG165 and pDPG480 which were subsequently selected on bialaphos-containing medium.

FIG. 8A. Leaf temperature data from Glufosinate® sensitive (mtlD negative) and resistant (mltD positive; low expressor) plants grown under water stress conditions in the field.

FIG. 8B. Leaf temperature data from Glufosinate® sensitive (mtlD negative) and resistant (mltD positive; high expressor) plants grown under water stress conditions in the field.

DETAILED DESCRIPTION OF THE INVENTION

The identification and characterization of plants that are resistant or tolerant to water deprivation has long been a goal of agronomy. However, it has not been possible to accomplish the identification and isolation of genes that can provide resistance or tolerance to water stress. The insertion of such genes into monocots has the potential for long term improvement in, and expansion of, agriculture world-wide.

The ability of a plant to adapt to changes in water and salt concentrations is dependent on the ability of the plant to osmotically adjust its intracellular environment by altering the concentration of osmoprotectants within the cells of the plant. These osmoprotectants include, but are not limited to, various sugar molecules, such as monosaccharides, disaccharides, oligosaccharides, polysaccharides, sugar alcohols, and sugar derivatives. Thus, to provide a plant that is tolerant or resistant to a reduction in water availability, a preselected DNA segment or "gene" or "transgene" encoding an enzyme which catalyzes the synthesis of a particular osmoprotectant can be introduced into the genome of the plant. The osmoprotectant may be one that is not normally synthesized by the plant, but one which can be synthesized from a substrate that is abundant in the cells of the plant after the introduction of the preselected DNA segment. In the alternative, the osmoprotectant may be one that is naturally synthesized by the plant but the levels of the osmoprotectant in the plant are insufficient to render the plant tolerant to a reduction in water availability.

The accumulation of a non-naturally occurring osmoprotectant in a plant, plant cell, plant part, or plant tissue, could result in a detrimental effect because the substrate employed to synthesize the osmoprotectant is being depleted and a non-naturally occurring product is produced, which most likely would not be degraded. Moreover, a single introduced preselected DNA segment in the

APPENDIX D
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Also provided is an expression cassette comprising a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of an osmoprotectant, operably linked to a promoter functional in a host cell, wherein a second DNA segment separates the first preselected DNA segment encoding the enzyme from the promoter. A preferred second DNA segment is the *Adh1* intron 1.

Further provided is an expression cassette comprising a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of an osmoprotectant, operably linked to a promoter functional in a host cell, wherein a second DNA segment encoding a maize chloroplast transit peptide is operably linked to the preselected first DNA segment encoding the enzyme.

As used herein, a "preselected" DNA sequence or segment is an exogenous or recombinant DNA sequence or segment that encodes an enzyme which catalyzes the synthesis of an osmoprotectant, such as a sugar. The enzyme preferably utilizes a substrate that is abundant in the plant cell. More preferably, the substrate is present in either, or both, the cytosol and chloroplasts of the plant cell. It is also preferred that the preselected DNA segment or sequence encode an enzyme that is active without a co-factor, or with a readily available co-factor. For example, the *mtlD* gene of *E. coli* encodes a mannitol-1-phosphate dehydrogenase (M1PD). The only co-factor necessary for the enzymatic activity of M1PD in plants is NADH and the substrate for M1PD in plants is fructose-6-phosphate. Both NADH and fructose-6-phosphate are plentiful in higher plant cells.

As used herein, "substantially increased" or "elevated" levels of an osmoprotectant in a transformed plant cell, plant tissue, plant part, or plant, are greater than the levels in an untransformed plant cell, plant part, plant tissue, or plant, i.e., one where the genome has not been altered by the presence of a preselected DNA sequence. In the alternative, "substantially increased" or "elevated" levels of an osmoprotectant in a water-stressed transformed plant cell, plant tissue, plant part, or plant, are levels that are at least about 1.1 to 50 times,

E4

Appendix E

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Thomas R. Adams et al.

Serial No. 09/732,439

Filed: December 7, 2000

Title: TRANSGENIC MAIZE WITH INCREASED MANNITOL CONTENT

Group Art Unit: 1638

Examiner: Benzion, G.

Atty. Dkt. No.: DEKM:184USD1
(formerly 950.030US2)

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on the date below.

06/26/02
Date

Robert E. Hanson

PRELIMINARY AMENDMENT

Hon. Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the application as indicated below.

In the Claims:

Please amend claims 64 and 82 as follows:

64. (Amended) A method for producing the plant of claim 59 comprising:

(a) introducing into cells of a monocot plant an expression cassette comprising a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in the monocot plant cells, to yield transformed monocot plant cells; and

(b) expressing the enzyme encoded by the preselected first DNA segment in the transformed monocot plant cells so as to render the transformed monocot plant cells substantially water stress tolerant or resistant.

82. (Amended) An expression cassette for producing the transformed monocot plant of claim 59, wherein the expression cassette comprises a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in a host cell, where in the promoter is selected from the group consisting of the *Glb* promoter, the *Adh1* promoter, and the *Act1* promoter.

COMMENTS

Claims 64 and 82 have been amended herein to clarify the relationship of the claims with the subject matter of claim 59. The amendments do not change the scope of the claims and, accordingly, Applicants do not intend to disclaim any subject matter by the amendments. A marked copy of the amendments is provided in **Appendix A**. Claims 59-96 are pending in the case and are presented herein for consideration. The Examiner is invited to contact the undersigned at (512)536-3085 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

Robert E. Hanson
Reg. No. 42,628
Attorney for Applicant

FULBRIGHT & JAWORSKI, L.L.P.
600 Congress Ave., Ste. 1900
Austin, Texas 78701
(512) 536-3085

Date: August 26, 2002

APPENDIX A: MARKED VERSION OF AMENDED CLAIMS

64. (Amended) A method [to increase water stress resistance or tolerance in monocot plant cells] for producing the plant of claim 59 comprising:

(a) introducing into cells of a monocot plant an expression cassette comprising a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in the monocot plant cells, to yield transformed monocot plant cells; and

(b) expressing the enzyme encoded by the preselected first DNA segment in the transformed monocot plant cells so as to render the transformed monocot plant cells substantially water stress tolerant or resistant.

82. (Amended) An expression cassette for producing the transformed monocot plant of claim 59, wherein the expression cassette comprises [comprising] a preselected first DNA segment encoding an enzyme which catalyzes the synthesis of the osmoprotectant proline, operably linked to a promoter functional in a host cell, where in the promoter is selected from the group consisting of the *Glb* promoter, the *Adh1* promoter, and the *Act1* promoter.